

Detection and Identification of *Dasheen mosaic virus* Infecting *Colocasia esculenta* in India

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Abstract Reverse transcription polymerase chain reaction of the infected leaf samples of *Colocasia esculenta* plants showing severe whitish featherly symptoms were carried out using *Potyvirus* group specific primers, resulting in an amplicon of 327 bp, encoding the core region of the coat protein gene. Sequencing and BLAST analysis showed that the virus is distinct, closely related to *Dasheen mosaic virus* (DsMV). Sequence analysis revealed 86 and 96% identity at the nucleotide and amino acid level respectively with the DsMV isolate SY1 (accession Number AJ628756). This is the first molecular level characterisation of the DsMV infecting *C. esculenta* in India.

Keywords *Colocasia esculenta* · *Dasheen mosaic virus* · Potyvirus · RT-PCR · Taro

Taro (*Colocasia esculenta*) a tropical tuber crop, belonging to the family of Araceae, is of great economic importance because of its use as a staple food, highly nutritious and good economic returns. It is grown primarily as a vegetable food for its edible corms which are very high in starch dietary fibers, and secondarily as leaf vegetables. *Dasheen mosaic virus* (DsMV) is one of the important viral pathogen of *C. esculenta*, which develops pale green feathering symptoms, or may have severe or slight vein-banding

symptoms or no visible symptoms at all. The virus causes variation patterns in colour, shapes, and sizes of the leaf and may reduce the corm yield. Symptom expression is often intermittent or seasonal. Alconero and Zettler [1] reported the alternation of symptomatic and asymptomatic leaves on the same *C. esculenta* plants.

DsMV first reported from Dasheen (*Colocasia esculenta* (L) Schott) [15], is an aphid transmitted potyvirus, found to infect a wide variety of cultivated aroids and ornamental plants worldwide. It causes serious damage to the ornamentals like *Caladium*, *Dieffenbachia*, and *Zantedeschia*, and is ubiquitous in commercial plantings of the tropical root crops, of the genera *Colocasia*, *Xanthosoma* and *Amorphophallus* [3]. Ram et al. [10] first time reported DsMV infection from four ornamental aroids in India. The virus is transmitted by several widely distributed aphid species, including *Myzus persicae* and *Aphis gossypii* in a non-persistent manner. Since, most of the commercially cultivated hosts are vegetatively propagated; the infection also spreads by infected planting material. DsMV being an economically important viral pathogen diagnosis of the virus is important, for the production of virus free planting materials.

In India, a mosaic disease on *Colocasia* has been reported from Thiruvananthapuram, with a typical symptom resembling that of DsMV [8]. Occurrence of mosaic disease on *Colocasia esculenta* in north eastern states of India has also been recorded [12]. However there has been no further study to isolate or identify the viral isolate infecting *C. esculenta*.

Previously serological techniques like ELISA have been used for the diagnosis of viruses, which seems to be more laborious, high cost and time consuming. Moreover serological diagnosis of potyviruses is often ambiguous, because of frequent serological cross-reactions between

Nucleotides sequences have been submitted to NCBI data base under the accession number: FJ160765.

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species [2] and biological indexing is very cumbersome. Conversely, recent advances in nucleic acid technologies have enabled the development of powerful detection and identification tools. Reverse transcription polymerase chain reaction (RT-PCR) based methods enable fast, accurate detection, quantification and characterization of potyvirus [7]. The present study describes about the detection and identification of the DsMV infecting *C. esculenta*, through cloning and sequencing of the partial coat protein gene of the virus.

Leaf samples of infected taro (*C. esculenta*), showing whitish feathery symptoms were collected from different locations of Kerala, Tamil Nadu, Andhra Pradesh and Karnataka, and total RNA was extracted from symptomatic (five samples from each location) and healthy leaves using the QIAGEN RNeasy plant mini kit (Maryland, USA), according to the manufacturers' protocol. The isolated RNA was subjected to RT-PCR using one step RT-PCR kit (Finnzymes, Espoo, Finland). The RT-PCR was performed in a 20 µl reaction mixture containing 5 µl RNA, 1 µl of 20 pmol of oligo d(T)₁₆, 1 µl of 20 pmol each of potyvirus group specific primers MJ1(f)-5'-TGGTHTGGT GYATHGARAAYGG-3' and MJ2(R)-5'-TGCTGCKGC YTTCATYTG-3' [9, 5], 2.0 µl of 10×/5× reaction buffer, 0.5 µl of 10 mM dNTPs, 0.5 µl of 50 mM magnesium chloride (MgCl₂), 0.25 µl of (5U/µl) AMV Reverse Transcriptase and 1.0 µl of (1U/µl) Dynazyme DNA Polymerase. The RT-PCR was performed in Eppendorf Mastercycler Gradient ES with the following thermal cycle programme: 48°C for 45 min, 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 50°C for 1 min, 72°C for 1 min and a final extension of 72°C for 10 min. The amplified product was analyzed on 1% agarose gel, stained with ethidium bromide and imaged using Alpha imager.

The amplified PCR products were gel purified using the Gel Extraction Kit (QIAGEN, USA), cloned into the pGEM-T Easy vector (Promega, USA) and transformed into the *E. coli* strain DH5α and the resulting recombinant clones were selected on LA medium containing ampicillin and X-gal/IPTG [11]. The clones were then subjected to automated sequencing to both directions using T7 and SP6 primers. The sequenced strands were then edited using the BIOEDIT Software [6]. The nucleotide and the deduced amino acid sequence were compared with those in the NCBI databases using the Basic Local Alignment Search Tool (BLAST; <http://www.ncbi.nlm.nih.gov/blast/>), with BLASTN and BLASTP respectively. Sequences from the accessions giving the highest and lowest match scores and closest matching sequence from a different species was considered. Multiple sequence alignment of the virus isolates and the percent homology between aligned sequences were calculated using Clustal W [14]. From the aligned sequences a phylogenetic tree was constructed using the

neighbor-joining method [13], the data sets were subjected to 100 bootstraps replicates and the tree was constructed using the MEGA software.

Infected leaf samples of *C. esculenta* collected from different locations showed a common pattern of infection such as whitish feathery symptoms and mosaic (Fig. 1). Among the symptoms, appearance of whitish feathery symptoms appears to be the most common ones. The feathery symptom appears to be mild, localised to some specific regions of the leaf either along the midrib, leaf marginal veins or appears to be severe infecting the entire leaf. The plants showed patterns of various colours, shapes and sizes on the leaf. Total RNA was isolated and stored in -20°C until further use.

One step RT-PCR of the total RNA isolated from *C. esculenta* leaves using MJ1 and MJ2 primers resulted in the amplification of 327 bp partial coat protein gene (Fig. 1). The primers amplified the partial internal region of the coat protein. The amplified product was cloned in pGEMT Easy vector, sequenced and the sequences deposited in the GenBank (accession number FJ160765). The sequences were edited using the BIOEDIT software and were used for further analysis. Comparison of the nucleotide sequence of the partial coat protein gene, using the BLAST showed a higher identity of the viral isolate under study with that of the *Dasheen mosaic virus*, isolate SY1 (Accession no.: AJ628756), with a similarity of 86%. The isolate SY1 has been reported from aroids, infected with DsMV from China. The nucleotide sequence showed similarity with all the other DsMV isolates, with a similarity ranging from 83 to 86%. The virus also resembled close similarity with *Vanilla mosaic virus*, a virus related to DsMV with a similarity of 85%. The translated amino acid sequence of the virus was also analysed using the BLAST, and found that the sequence appears to be highly similar among all the DsMV isolate, with a similarity of greater than 90%. Among the DsMV isolates, *C. esculenta* viral isolate shows a higher similarity with the isolate SY1 and a least similarity with the *Dasheen mosaic virus*- VN/Ce2 (Accession no.: DQ925465). The viral isolate under study also showed much higher similarity with the *Vanilla mosaic virus*, stating that the two viruses are closely related. The phylogenetic tree constructed using the neighbour-joining method of the MEGA Software with 100 bootstrap replicates and with *Vanilla mosaic virus* as the out group, showed a closed similarity of the isolated virus under study to that with DsMV, isolate SY1 (Fig. 2).

The group specific PCR and subsequent molecular analysis of amplified regions has been used for rapid detection and identification of potyviruses and is appeared to be most suitable method for identification of viruses, difficult to purify and/or occurring in mixed infections [4]. Compared to serological studies, the molecular detection

Fig. 1 *Colocasia esculenta* leaf samples showing whitish feathery mosaic symptoms and RT-PCR analysis of mosaic affected *Colocasia esculenta* plants with potyvirus group specific primers MJ1 and MJ2. **a** Mosaic and severe whitish feathery symptom, **b** and **c** Whitish feathery symptom, lane M 100 bp marker, lane 1–10 Infected *Colocasia esculenta* leaf samples, lane 11 Healthy plant

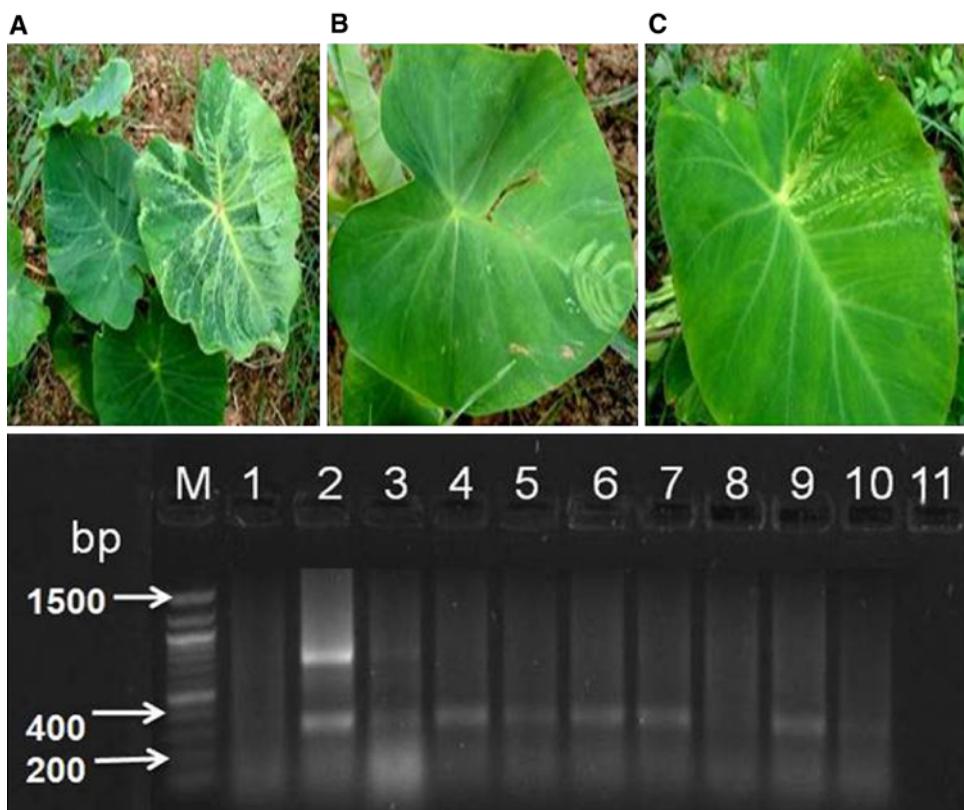
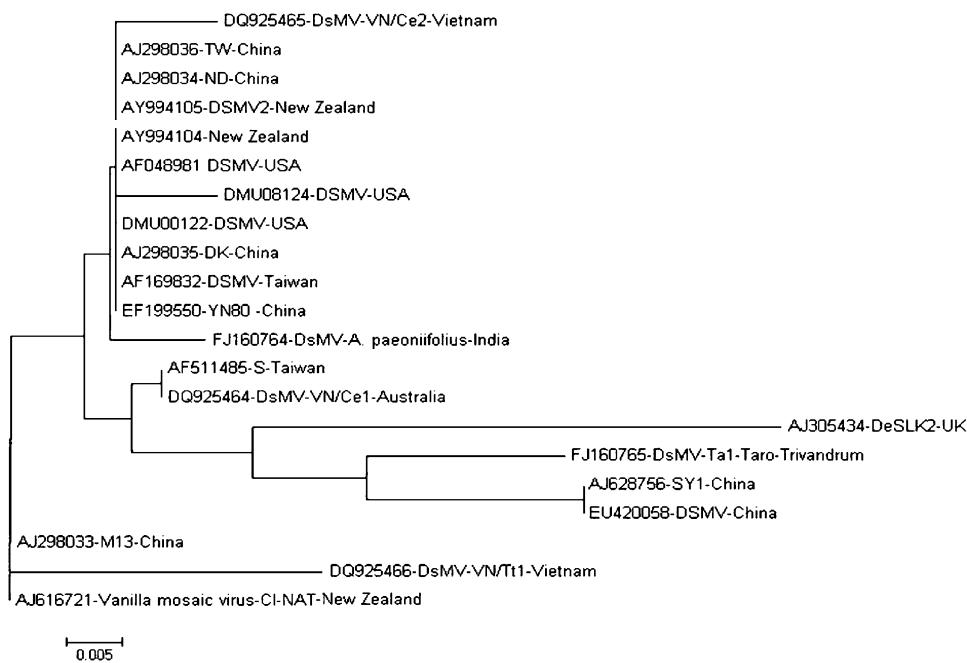


Fig. 2 Phylogenetic tree illustrating similarity of DsMV infecting *Colocasia esculenta* with different viral isolates using neighbor-joining method with 100 bootstrap replicates and with *Vanilla mosaic virus* as the out group



methods would facilitate the epidemiological studies to be performed on *C. esculenta* infected by viruses of the members of the genus *Potyvirus*, particularly DsMV. On the basis of sequence identification and cluster analysis, using these short amplicon of 327 bp were found sufficient

to detect and identify the virus species. Thus RT-PCR with MJ1 and MJ2 primers, designed to amplify a short conserved region of the potyviruses seems useful for the detection and identification of the potyvirus infecting *C. esculenta* in India.

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